PLANT GENETIC MECHANISMS

Panel Manager - Dr. Robert J. Ferl, University of Florida
Program Director - Dr. Liang-Shiou Lin

This program area supports studies addressing the basic cellular, molecular, genetic and cytogenetic processes which contribute new information required for the development of novel approaches to crop and forest improvement. Innovative research is emphasized in the following areas: (1) characterization of agriculturally important genes and gene products, (2) relationship between gene structure and function, (3) regulatory mechanisms of expression of nuclear and organellar genes, (4) interactions between nuclear and organellar genomes, (5) mechanisms of recombination, transposition, replication, and repair, and (6) epigenetic mechanisms that influence gene expression.

2000-01461 Tobacco Chloroplast DNA Replication Origins and Origin-Binding Proteins

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Grant 00-35301-9107; $100,000; 2 Years

In plants, photosynthesis occurs in chloroplasts. Chloroplasts contain DNA that encodes genes essential for photosynthesis and other chloroplast functions. Much effort is being expended to characterize gene expression in chloroplasts, and there are growing efforts to express introduced genes in chloroplasts to enhance plant productivity. However, to fully make use of chloroplast gene expression for the improvement of plants, the mechanism by which chloroplast DNA replicates and is partitioned to new chloroplasts during plant growth needs to be understood. Much of the interest in chloroplast genetic engineering is based on the very high genome copy number that is present in certain leaf cells at specific periods of development. However, chloroplast genome copy number per cell varies extensively at different stages of plant development, and while there are theories as to the role this variation may play, the mechanism for it is not known. The experiments outlined in this project will provide a better understanding of the sequences and proteins involved in chloroplast DNA replication and genome copy number regulation. Using *in vitro* DNA replication assays, we specifically plan to analyze deletion mutants to determine the minimal DNA sequences required for functional chloroplast DNA replication. We will characterize chloroplast proteins that bind to the minimal origin regions, and determine the role of these proteins in replication *in vitro* and *in vivo*. Results from this work will contribute to our understanding of chloroplast development and how it relates with photosynthesis, and may lead to better vectors and/or strategies for chloroplast genetic engineering.

2000-01494 Analysis of Genetic Relationships Between Ryegrass (*Lolium* sp.) Biotypes and the Spread of Diclofop-Resistant Ryegrass in the Southern Region

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Strengthening Award; Grant 2001-35301-09970; $100,000; 2 Years

Ryegrass (*Lolium* sp.) is a major weed problem in wheat production. Diclofop is a chemical that can kill seedlings of annual grasses, including ryegrass, but will not injure wheat.

Wheat producers have used Diclofop to control ryegrass since the 1970's. When a weed population is repeatedly exposed to the same herbicide, survivors from each spray event will eventually produce plants that will not be killed by the recommended herbicide dose. Such plants have developed resistance to the herbicide. In the United States, Diclofop-resistant ryegrass has now been reported in nine wheat-producing states. This project aims to determine whether the resistant ryegrass populations in Arkansas and Missouri developed separately or were also spread by seed. Understanding the spread of resistance would help develop effective management strategies for diclofop-resistant ryegrass. To determine the relatedness of resistant populations, DNA “fingerprints” from a sample of each population will be generated using a technique called amplified fragment length polymorphism (AFLP). The same genotypes will produce the same fingerprints. Another goal of this project is to identify part of the plant’s DNA that is unique among resistant individuals. Confirmation of resistance is currently done, by growing plants from seed and spraying the seedlings with diclofop. With a DNA marker, identification of resistant plants can be done faster. Bulk segregate analysis will be used to identify DNA markers associated with diclofop resistance. Homogeneous resistant and susceptible samples will be used. Other resistant samples will then be tested for the presence of such markers.

2000-01492 Comparison of Antisense and Sense Transgene-Mediated Suppression Mechanism(s)

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Postdoctoral Fellowship; Grant 2001-35301-09895; $89,992; 2 Years

Homology-dependent gene silencing occurs when the introduction of extra copies of a gene of interest into the genome of higher plants results in decreased expression of both the introduced and original genes. The gene of interest can be introduced in either the correct (sense) orientation or the opposite (antisense) orientation. In both instances, decreased protein expression is mediated through RNA degradation, suggesting a common mechanism of action. However, in petunia, the flower color patterns produced by either a single introduced sense or antisense chalcone synthase gene are distinctly different. The objective of this proposal is to determine whether these observed differences can be attributed to different mechanisms of action or whether a shared mechanism occurs in different cellular locations. In order to test whether sense and antisense gene silencing mechanisms share common components, mutations resulting in a loss of silencing will be generated in an antisense suppressed petunia line. Once non-silenced mutant plants are identified, the antisense gene will be "flipped" to a sense orientation and screened for similar effects on gene silencing. In addition, cellular localization of gene silencing will be examined by the disruption of RNA transport out of the nucleus for both the introduced sense and antisense chalcone synthase genes. Examining pathways involved in gene silencing will lead to an improved understanding of fundamental questions involving control of gene expression in plants. This knowledge can eventually be used to improve methods currently utilized in manipulating gene expression of genetically engineered agricultural plants.

2000-01471 Protein Tyrosine Phosphatases and Their Function in Higher Plants

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Grant 98-35301-6172; $120,000; 2 Years

Protein tyrosine phosphatases (PTP) play a key role in growth control and stress response in mammalian and yeast systems. However, none of these enzymes has been characterized in higher plants until recently when we identified the first plant PTP from *Arabidopsis* (AtPTP1). Following the molecular studies, we began to dissect the function of AtPTP1 by biochemical and molecular genetics methods. We have found some interesting changes in the plants that produce less AtPTP1 activity. Our further studies will focus on the mechanisms underlying the function of AtPTP1 in plants. The first objective is to isolate and characterize AtPTP1 "knockout" mutants, plants that do not produce any AtPTP1 protein. We expect to see some major changes in the growth and development of these mutant plants. The second objective is to understand how AtPTP1 functions in plant cellular processes. One of the proteins that PTP1 regulates is a group of protein kianses called MAPK. If a mutant *Arabidopsis* lacks AtPTP1, this plant should produce more MAPK activity because AtPTP1 inhibits the MAPK activity. Our studies on plant PTP will bridge the gap in our knowledge about PTP in eukaryotic systems. From the viewpoint of basic science, further molecular studies may provide a link between PTP function and plant developmental processes. On the practical side, our findings may provide an exciting new mechanism to regulate growth and yield of crop plants.

2000-01474 The Positive Effect of Introns on Plant Gene Expression

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Grant 00-35301-9082; $210,000; 3 Years

The information contained in most plant genes is interrupted by non-coding sequences known as introns. Introns are deleted after the gene has been transcribed into mRNA but before the mRNA is translated into protein. Introns are not simply a kind of packaging material that must be removed before the information in a gene can be read. In many examples, the amount of protein derived from a gene is greatly increased by the presence of an intron. Currently, the means by which introns elevate expression are not known. The goal of this project is to determine the mechanistic basis for the positive effect of introns on the expression of plant genes. Experiments to achieve this goal will be performed with a well characterized and versatile test system consisting of a gene that has been modified to facilitate the quantification of gene expression, and one of that gene's natural introns which can be easily isolated and experimentally manipulated. The aims of this research are to determine which structural features of introns are required to increase expression, and to explore possible biochemical differences in the mRNA derived from genes containing or lacking introns. This research will increase our understanding of a very important but poorly understood fundamental aspect of plant gene expression. A deeper understanding of the factors needed for abundant gene expression could have great practical benefit because there are many scientific, agricultural, and commercial applications in which a high level of protein synthesis is desirable.

2000-01505 3' Modification of Plant Mitochondrial mRNAs

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Grant 00-35301-9109; $100,000; 2 Years

Mitochondria are semi-autonomous organelles that have a genome that partially encodes the functions of the organelle. Mitochondrial genes are expressed through prokaryotic (or bacterial-like) mechanisms, and gene expression is regulated through novel mechanisms. Plant mitochondria use several unusual RNA processing reactions in the expression of mitochondrial genes including RNA editing, *trans*-splicing of introns, and polyadenylation. We have recently discovered a novel 3' modification of plant mitochondrial mRNAs in which one or several nucleotides may be added to the 3' terminus of plant mitochondrial mRNAs ("short modification"). The most abundantly modified mRNAs detected are characterized by having a high rate of turnover and are modified near the codon, apparently after RNA cleavage reaction. Three prime polyadenylation has recently been reported to plant mitochondrial mRNAs, and may play a role in regulating RNA turnover. The objectives of this research proposal will be to: carefully examine the nature and occurrence of polyadenylated and short modified transcripts in terms of the relative abundance of these transcripts; and to examine the relationship of these modifications to RNA stability, both *in vivo* and *in vitro*. This will establish the roles of these reactions in RNA metabolism. We will characterize the RNA processing reactions (polyadenylation and short modification), and the interaction of modified and unmodified RNAs with the RNA turnover apparatus *in vitro*.

2000-01464 The Role of Ribosome Heterogeneity in the Regulation of mRNA Translation
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Grant 00-35301-9108; $ 210,000; 3 Years

The synthesis of proteins in cells is an absolute requirement for life In seed crops, such as corn, the level of protein synthesis during the kernel development is an important determinant of yield. In all plants, environmental stress results in reduced levels and altered modes of protein synthesis. Our goal is to understand mechanisms of protein synthesis in order to control the production of specific proteins and to increase levels during critical times of development and in adverse environments. Protein synthesis is carried out by the ribosome, a cell organelle with two subunits composed of about ninety proteins and four RNAs, with the assistance of a large number of proteins called translation factors. Protein synthesis occurs when a messenger RNA (mRNA) transcript is selected and decoded by the ribosome to construct a polypeptide (protein) chain. This process is regulated at a global level and through the competition between mRNAs for translation. We will study the role of a group of proteins of the large subunit of the ribosome, the P-proteins, which form a complex involved in polypeptide chain formation. Levels of the P-proteins vary during development and their phosphorylation is altered by stress. We will study the effect of mutations of these proteins in cell extracts and in whole plants. We will evaluate how this protein complex influences the interaction of an important translation factor with the ribosome. These studies will provide valuable information on the role of ribosomes in the regulation of protein synthesis.

2000-01490 Molecular Analysis of HSP101-Mediated Translational Enhancement
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Grant 00-35301-9086; $253,000; 3 Years

A long standing goal of agriculture has been to increase the protein content of crop species. An informed approach towards improving plants, particularly crop species, requires not only understanding the role of genes but how their expression is controlled. An integral part of this approach requires an understanding of translation and how it is regulated. We have demonstrated that several genes contain a translation enhancer that increases protein synthesis. We have demonstrated that the heat shock protein, HSP101, is responsible for the increase in translation protein synthesis. Genetic studies using yeast suggested that two factors involved in protein synthesis, i.e., eIF4G and eIF3, were required for the HSP101-mediated regulation. Although eIF4G is a single protein, eIF3 is composed of approximately 10 subunits. We will investigate whether HSP101 physically interacts with eIF4G and identify which subunit of eIF3 that HSP101 interacts with. We will determine what part of the HSP101 protein is responsible for the interaction. We will also determine the expression patterns of HSP101 in crop species such as corn. We will also examine other genes in plants to determine whether protein synthesis from these genes may be regulated by HSP101.

2000-01512 Organelle Containing Neutral Lipids and Unique Proteins in Anthers

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Grant 00-35301-6037; $140,000, 2 Years

Modulating sexual reproduction in crops is important to productivity. Promoting sexual reproduction could enhance the yield of fruits and seeds. Suppressing the process could increase the production of vegetable produce. Other possible manipulations include utilizing male sterile genes in the production of hybrid seeds, generating seedless fruits, and eliminating self-incompatibility. A major step in sexual reproduction is the interaction between the pollen (containing the male sex cell) and the female part (containing the female egg) of the flower. An essential component in the interaction is the pollen-coat materials, which are present on the pollen surface. They are derived mostly from a layer of cells, called the tapetum, surrounding the pollen sac in the pollen-producing flower. *Brassica* (the cabbage family) has many economically important species, including many vegetables and the oil-producing canola. *Brassica* pollen coat contains special proteins (termed oleosins) and lipids (steryl esters), which were initially accumulated in two abundant subcellular particles (organelles) in the tapetum cells. One of the organelles is the tapetosome, which contains oleosin, triglycerides, and massive vesicles. Only the oleosin will be preserved and transported to the pollen coat. The other organelle is the non-green plastid (elaioplasts = oil plastids), which contains abundant steryl esters in small globules. Only the core steryl esters will be preserved and transported to the pollen coat. I propose a study of the structure, function, synthesis and degradation of the tapetosomes. The organelles will be subfractionated and their structures and chemical constituents will be assessed. The synthesis and degradation of the individual constituents will be analyzed. The oleosins on the pollen surface will be removed to test if the proteins act as a wick to draw water from the female structure of the flowers for pollen germination. The above studies will be aided by using oleosin-less pollen produced by genetic means. I also propose similar studies on the structure, synthesis, and degradation of the elaioplasts, especially the globules. The control of the synthesis of the structural proteins on the globules in the elaioplasts will be studied by characterizing their genes and expression. The overall information will be used to design strategies for the manipulation of the sexual reproduction process in *Brassica* and other agricultural crops.

2000-01488 Intron Enhanced Gene Expression in Maize

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Grant 00-35301-9119; $210,000, 3 Years

Genes contain intervening sequences (introns) which do not encode protein information and are spliced from the primary RNA transcripts. Certain introns have the ability to increase gene expression when included in the upstream portion of the transcriptional unit, but the mechanisms of enhancement are still unknown. Information to date indicates that this positive effect is likely post-transcriptional, and there may be varied modes of action for different introns. For example, enhancing introns vary greatly in the extent of stimulation. Among plant introns, one of the most effective is the first intron of the maize *shrunken1* (*Sh1)* gene. This intron enhances transient expression up to 100-fold in cultured cells of several monocots. A more complete understanding of how introns function has important agricultural applications for the optimization of gene expression. We propose experiments to elucidate the mechanisms of intron-mediated enhancement and to determine whether the intron functions in transgenic maize as it does in cultured cells. We have demonstrated that intron splicing is essential for enhancement, and will determine whether the splicing process facilitates transcript maturation, transport, or recognition for protein synthesis. A *Sh1* sequence motif required for maximum enhancement but not for splicing will be tested for its ability to convert weakly or non-stimulating introns into more robust enhancers of expression. The *Sh1* first intron is now being used in agriculturally relevant transgenics. An understanding of how it modulates gene expression is directly relevant to extant and future plant improvement programs.

2000-01460 The Role of Transposable Elements in the Evolution of Polyploid Cotton

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Grant 00-35301-9118; $140,000; 2 Years

Our long-term goal is to understand the impact of transposable elements (TEs), also known as “jumping genes,” on the DNA of plants that are 'polyploid,' combining multiple sets of chromosomes from different ancestors. Cotton is an elegant model for study of polyploidy, an evolutionary mechanism that partly accounts for productivity of many major crops. Previously, the PI's lab isolated members of 83 transposon-like DNA families accounting for 24% of cotton's DNA. Detailed characterization of a subset of these families will determine if they actually are transposons, and identify their key features. This information will permit us to pursue our main goal, to study the levels and patterns of 'jumping' of these elements during the course of cotton evolution. Two new techniques, one developed in the PI's lab (the 'BAC-RF' method) and the other developed in the co-PI's lab ('transposon display'), make this work possible. This work will create new avenues needed to provide intrinsic genetic solutions to challenges facing the $6 billion/year U.S. cotton industry, and will train young scientists in new approaches to crop improvement. In addition to a high yield of basic information, some likely applied spinoffs of this work will include (1) the development of an efficient new system for DNA fingerprinting of cotton, useful for accelerating breeding progress and also defining the results of private-sector research investments; and (2) progress toward development of new systems for cloning of cotton genes that are directly related to key aspects of cotton quality and productivity.

2000-01478 An Aldehyde Dehydrogenase Required for Male Fertility in Maize
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Grant 00-35301-6545; $176,000, 2 Years

Cytoplasmic male sterility (cms) is a maternally inherited inability to produce functional pollen and is a widespread phenomenon in the plant kingdom. In many cms systems, restoration of male fertility can occur in the presence of specific nuclear genes, termed restorers. Because cms and nuclear restorer systems play important roles in hybrid seed production, an understanding of the molecular mechanisms that lead to cms and fertility restoration has direct relevance to US agriculture. Using prior USDA support, a nuclear restorer of cmsT maize was cloned. This gene (*rf2*) is the only restorer cloned to date. As such, it provides a unique resource for the study of the molecular mechanisms associated with fertility restoration in cms systems. In addition to its importance in fertility restoration of cmsT maize, this gene is also required for male flower development in normal maize. The protein produced by the *rf2* gene is an enzyme that acts on a class of chemicals termed aldehydes. Coordinated biochemical and genetic experiments designed to determine the specific metabolic function(s) of the RF2 protein will be conducted. In combination, these experiments will enhance our understanding of both the mechanism by which fertility restoration occurs in cmsT and the role of basic metabolic processes in normal flower development.

2000-01448 Genes and Regulatory Systems Controlling Adaptation to Changes in Light Quality
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New Investigator Award; Grant 00-35301-9140; $210,000; 3 Years

For all photosynthetic organisms, the ability to perceive and respond to colors of light that are present in their environment is critical for survival. Yet very little is known about molecular mechanisms used by these organisms to adapt to changing conditions of light color. The study of such processes in higher plants is made difficult by the complexity of their sensing and regulatory systems. Thus we use cyanobacteria, evolutionary ancestors of higher plants, as a model system to understand more complex processes that are occurring in higher plant systems. Our project will establish a basic understanding of the global cellular transcript accumulation responses that occur during changes in light quality through the use of new technology for genome-wide analysis of mRNA levels. This technology, called DNA microarray analysis, allows the simultaneous measurement of the expression levels of essentially all of the genes in an organism's genome. A powerful aspect of working with a simpler cyanobacterial model system is that both the size of the genome and number of genes contained within it are 10 to 100 times smaller than for higher plants. This feature allows us to conduct whole genome DNA microarray studies on cyanobacteria, which is not yet possible for even the simplest plant model organism such as *Arabidopsis*. We will conduct our initial studies by further examining a well characterized light quality response called complementary chromatic adaptation. This red-/green-light photoreversible response controls many cellular processes, including light harvesting protein gene expression, through a novel prokaryotic phytochrome-like photoreceptor.

2000-01479 Genetic Analysis of Postmating Reproductive Barriers in Tomato
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Postdoctoral Fellowship; Grant 2001-35301-09971; $89,712; 2 Years

Plant breeders use hybridization between unimproved wild and elite material to introgress favorable traits into the germplasm pool of a crop. This transfer is mainly limited, however, to crosses that are easily achieved between closely-related sources. Reproductive barriers erected during the process of speciation between diverging groups prevent wider crosses by effects ranging from complete lethality to reduced viability in recombinant genotypes. An explanation of this phenomena proposed by Muller and Dobzhansky during the neo-Darwinian synthesis involves interacting pairs of genes evolving independently in the different lineages. These interactions become fine-tuned between the pairs of genes in each lineage, a process called coadaptation, such that when they are brought together again through hybridization, only offspring with parental or ancestral combinations survive. While deleterious effects in some interspecific crosses have long been recognized, we still know very little about the underlying genetic basis. This proposal will use the model vegetable, tomato, to investigate one example of reproductive isolation. Briefly, we will generate a large cross between *Lycopersicon esculentum*, the garden tomato, and *L. pennelli*, a wild relative, to look for combinations of genes absent in the progeny. The locations of these factors will be compared with the locations of genes affecting pollen viability, fecundity, self-incompatibility, and morphological traits important in the divergence of these two species. This will be a first step towards isolating and characterizing the coadapted gene complexes operating in this cross, and may suggest whether these genes are a cause or byproduct of speciation.

2000-01268 Regulation of Trans-gene Expression in Wheat

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Seed Grant; Grant 00-35311-9363; $74,999; 2 Years

Wheat, the most widely cultivated crop in the world, is a basic staple of our diet. The long term goals of this project are to improve wheat by introducing non-native genes (trans-genes) into wheat. We propose to use trans-genes with regulated expression to enhance wheat nutritional value and environmental tolerances. This will be of value to US agriculture and to human nutrition in general. To accomplish these goals we developed a protocol to introduce trans-genes into wheat immature embryos and embryogenic callus using the soil bacterium *Agrobacterium tumefaciens* (Peters, Ackerman, and Davis, *Plant Molecular Biology Reporter* 4*:* 323-331, 1999). The DNA transferred by *Agrobacterium* to wheat is part of a plasmid, which we have also modified by adding a modular insert. The insert facilitates exchange of different promoters, protein coding regions, and 3' untranslated sequences. The transgenic protein can also have FLAG and $(His)_6$ epitope-tags to aid in biochemical purification of the proteins. Our goals are to (1) optimize gene expression in our new wheat transformation system. We will use a gene that is required for all RNA synthesis, the wheat general transcription protein *TATA Binding Protein.* These studies will establish the requirements for regulated systemic and tissue specific expression in wheat. Additionally, (2) wheat cells that have been transformed will be established in tissue culture as suspension cell lines. These cells will express epitope-tagged TATA binding protein in order to better isolate naturally occurring regulatory complexes containing TATA binding protein associated factors (TAFs) and preassembled RNA synthesis enzymes.

2000-01477 Homologous Recombination in Plant Mitochondria

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Grant 00-35301-9080; $102,000; 2 Years

In plant mitochondria, recombination between DNA repeats is generally tolerated. However, on occasion, very short repeats recombine, resulting in the elimination of an essential gene or the creation of a hybrid gene, with disastrous consequences. In those cases, normal gene expression is disrupted and the mitochondria fail to function adequately in the production of energy. As a consequence, plants experience developmental disturbances, which may include cytoplasmic male sterility and pollen abortion, white sectors, wrinkled leaves, or stunted growth. The process of homologous recombination is evolutionarily conserved and assumed to be responsible for the mitochondrial DNA rearrangements, but many of the debilitating rearrangements occur between extremely small repeats, which would be too short to mediate homologous recombination in bacteria. The research proposed here is designed to test the hypothesis that homologous recombination is responsible for the debilitating mitochondrial DNA rearrangements. To assess the importance of homologous recombination, transgenic plants will be produced, which contain the gene for the *E.coli* RecA protein, an enzyme essential for homologous recombination in bacteria. The protein product will be engineered for import into the mitochondria. By testing overexpression of both the wild-type *E.coli* RecA protein and a mutant version of the protein that blocks recombination, the consequence of stimulating and inhibiting homologous recombination in plant mitochondria will be investigated. This research will answer a basic question about the mechanism responsible for the debilitating mitochondrial DNA rearrangements. In addition, should homologous recombination be shown to be responsible, this investigation would suggest a procedure that could be adapted to produce cytoplasmic male sterile lines in other plants. Since cytoplasmic male sterility is an agronomically useful trait for seed production and plant breeding, the next step would be to apply the procedure to plants of agricultural importance.

2000-01491 Control of mRNA Stability in Dicotyledonous Plants

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Grant 00-35301-9681; $140,000; 2 Years

The long-term goal of this project is to elucidate the fundamental principles that govern mRNA stability in dicotyledonous plants. We are particularly interested in rapid decay mechanisms because these mechanisms can facilitate rapid responses to exogenous and endogenous stimuli. This project focuses on a rapid mRNA decay pathway in plants that is triggered by an RNA sequence element known as DST. We recently isolated the *dstl* mutant of *Arabidopsis* which is defective in DST-mediated mRNA decay. Our primary goal will be to further characterize this mutant to gain insight into the potential role of the *DSTI* gene product. To this end we plan to 1) continue experiments with a 600 element microarray to identify transcripts that change in *dstl* relative to wild type; 2) Determine, for the most interesting changes, if they reflect changes in mRNA stability; 3) Extend the resolution of the *dstl* map position relative to flanking markers to provide the foundation for gene cloning. Because no other selections for trans-acting sequence-specific mRNA decay mutants have been reported from eukaryotes, the information gained from studying *dstl* could have general significance. Beyond its contribution to basic knowledge, this project should suggest solutions to practical

problems resulting from the instability of some foreign mRNAs produced in plants for crop improvement.

2000-01149 Single Kernel Characterization System Equipment Grant
Giroux, M.J.; Habernicht, D.K.; Bruckner, P.L.; Talbert, L.E.
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Equipment Grant; Grant 00-35311-9340; $24,900; 1 Year

Wheat *(Triticum aestivum L.)* kernel size and endosperm texture are primary determinants of both milling and end-product quality. Not only are mean values for kernel size, kernel weight, and endosperm hardness important descriptors of end-use quality but also the variability within a given grain lot is directly related to consistency of wheat end-use quality and potentially milling performance. A single kernel characterization system (SKCS) developed by the USDA and Perten Instruments funded by the USDA will enhance research capabilities at Montana State University involving applied breeding objectives, applied agronomic and crop management research, and basic and applied cereal quality research objectives. The SKCS system evaluates, over a series of 300 individual kernel measurements, kernel moisture, kernel weight, kernel diameter, and kernel hardness. The SKCS equipment, to be housed in the Montana Cereal Quality Laboratory, will complement existing equipment primarily used for bread and noodle end-product evaluation. Primary uses of the SKCS system will be: by the Cereal Quality Laboratory for varietal characterization and collection of experimental data from agronomic and crop management research studies; by the wheat breeding programs to classify segregating breeding lines for endosperm hardness and to select for higher and more uniform kernel size; and by the cereal quality research program for basic research centered on the puroindoline genes a and b which affect wheat hardness and potentially other quality traits such as milling performance, starch damage, and water absorption. Early selection for SKCS grain quality parameters has the potential to enhance milling yield, baking absorption and quality.

2000-01498 Identification of Signaling Components in NPR1-Independent Resistance
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Grant 00-35301-9083; $270,000; 3 Years

The long-term goal of this project is to understand the mechanisms of induced disease resistance in plants. Previous work showed that the *NPRI* gene of *Arabidopsis thaliana* is a key regulatory component in transducing the salicylic acid (SA) signal during the onset systemic acquired resistance (SAR). Epistasis analysis performed in my laboratory using the gain-of-resistance *cpr* mutants and the loss-of-resistance *nprl* mutant clearly demonstrated the existence of an NPRl-independent signaling pathway leading to resistance to pathogens. In this proposal, we have designed two sets of experiments to further identify the signaling components in this NPRl-independent pathway. We will first focus on examining the function of NPRl-homologs, mainly NPR2 because of its high homology to NPR1, its induced expression during pathogen infection and the availability of a *npr2* mutant. The possible involvement of NPR2 in resistance will be assessed through a detailed study of its expression pattern and through characterization of the *npr2* mutant. In addition to focusing on NPR2, we also propose a broad search for mutants that block resistance to *P. parasitica* Noco2 in the *cpr5nprl* background. We are confident that this proposed research will lead to many interesting discoveries in the mechanisms of plant disease resistance. Better understanding of plant disease resistance will enable us to enhance

plants' own defense against diseases to reduce crop yield loss and lower the use of pesticides which are hazardous to the environment and human health. It is evident that this research may have a direct, positive impact on agriculture.

2000-01506 The Role of Nuclear-Encoded Sigma Factors in Maize Chloroplast Development

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Grant 00-35301-9016; $210,000; 3 Years

Chloroplasts in the leaves of maize develop from simple proplastids, found in cells at the base of the leaf, into photosynthetically active organelles in cells at the leaf tips. During this development process there are strictly orchestrated changes in expression of genes encoded by the chloroplast DNA. We seek to understand how these developmental changes in chloroplast gene expression are regulated. It is known that the transcription machinery of plastics is a major target of this regulation, and that nuclear-encoded proteins are involved. The chloroplast transcription enzyme (PEP RNAP) must form a complex with nuclear-encoded sigma factor proteins to transcribe specific sets of genes. Therefore, a possible mechanism mediating the developmental change in gene expression is production of different sigma factors in proplastids compared to chloroplasts. Assembly of PEP RNAP with different sigma factors could then drive the enzyme to transcribe different subsets of genes in the proplastid compared to the chloroplast. In support of this model, we have data showing that two sigma-like factors in maize accumulate differentially during chloroplast development. Our overall project goal is to investigate the role of these two nuclear-encoded proteins in chloroplast development and function. We will determine whether the two sigma-like factors assemble with PEP RNAP, whether they recognize different promoters, and whether they contribute to normal chloroplast development and function. These experiments will improve our understanding of general transcription mechanisms in plastids, thereby enhancing our ability to exploit the plastid compartment for engineering plants with value-added traits.

2000-01509 Mechanisms and Control of Rapid Genomic Changes in Flax

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Grant 00-35301-9079; $120,000; 2 Years

The genomes of many organisms may reorganize themselves on facing adversity, for which they are unprepared, in order to ensure the organism's survival. However, in spite of the recognition of the presence of innate systems that are able to restructure the genome, we know little about how the cell senses adversity and initiates its remarkable responses. A reproducible genomic destabilization has been shown in flax in response environmental stresses in the growing environment. The resulting alterations in the DNA are associated with defined genetic variants. One of the DNA sequence variants is a novel form of an insertion-like sequence. The objectives of this project are to understand the mechanism(s) by which the DNA changes occur, the timing of these changes and the genes responsible for the ability to respond to these environmental cues. The characterization of these labile regions will shed light on the type of mechanisms involved in the generation of these rapid genetic changes. These DNA markers will subsequently be used to isolate the genes that control the ability to respond. A related type of genomic instability has been widely observed in plants obtained from tissue culture. Plant tissue

culture is used extensively in plant biotechnology, and *in vitro* plant micropropagation is an important part of the agro-biotechnology industry. Plant tissue culture also has a crucial role to play in providing the target cells for modern genetic engineering approaches. The development of this set of markers will be vital for ensuring the genomic integrity of transgenic crops.

2000-01499 Characterization of Proteins Involved in Chromosome Cohesion in *Arabidopsis*
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Grant 00-35301-9341; $100,000; 2 Years

This study involves the cellular and molecular characterization of two proteins (SMC1 and SMC3) that participate in chromosome cohesion during meiosis and mitosis. Studies to determine how these proteins participate in chromosome cohesion will be conducted. We will isolate and characterize T-DNA tagged lines of *Arabidopsis thaliana* that are defective in the expression of SMC1 and SMC3 and conduct localization studies to determine when and where the proteins associate with chromosomes and identify proteins that interact with SMC1 and SMC3 to facilitate chromosome cohesion. Results obtained from these studies will provide important new insights into the processes of chromosome condensation and sister chromatic cohesion and a better understanding of how these two important events are linked. This information will provide new insights into early steps of pollen development, which could be utilized in the development of new male-sterility systems. Male sterile lines have been used extensively in seed production because they allow the mass production of hybrid seed without the labor-intensive and tedious process of hand emasculation of female seed parents. In addition, the lack of chromosome pairing during meiosis plays an important role in sterility associated with interspecific crosses. There are numerous traits in plants and animals that hold agricultural promise if they could be transferred to other species. However, the inability of "non homologous" chromosomes to pair and recombine during meiosis after interspecific crossing often results in sterility. Detailed knowledge of these processes may allow these barriers to be circumvented and facilitate gene transfer by interspecific crossing.

2000-01510 Molecular Mechanisms of Geminivirus Replication
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Grant 00-35301-9084; $260,000; 3 Years

Geminiviruses are serious pathogens of food, vegetable, and fiber crops in subtropical and tropical areas, including the Southeastern and Southwestern United States. A working knowledge of how these viruses replicate is necessary if we are to understand how they interact with their hosts to cause disease. Once they are identified and characterized, it should be possible to block critical interactions between viral and cellular proteins, destroying the viruses' ability to replicate. Thus, studying geminivirus replication mechanisms positively contributes to the improvement and sustainability of US agriculture. This research concerns mechanisms used by tomato golden mosaic virus (TGMV) to regulate its gene expression programs. TGMV AL2 protein (TrAP) is a transcription factor that stimulates expression of the viral coat protein gene (CP) by both activation and derepression mechanisms. However, TrAP does not bind the multiple DNA sequences in the CP promoter that are known to mediate its activity. Instead, we hypothesize that cellular proteins interact with TrAP and direct it to the regulatory sequences in

the CP promoter. Once tethered to the promoter, TrAP then interacts via its activation domain with other cellular proteins (general transcription factors; GTFs) that stimulate CP gene expression. Using genetic and biochemical methods, we intend to identify these cellular proteins and characterize their interactions with TrAP. In addition, the structure of TrAP itself will be investigated so that the nature of its interactions with cellular proteins that direct it to the CP promoter, and with cellular GTFs, can be better understood.

2000-01465 Mechanisms of LLS1 Cell Protective Function in Plants
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Grant 00-35301-9276; $120,000; 2 Years

Plants have evolved many mechanisms by which to cope with environmental stress but these have only begun to be understood at the molecular level. By investigating the genes involved in maintaining cell viability in the face of attack or damage by biotic and abiotic factors (i.e., disease and weather) it will be possible to understand what determines the robustness of a plant. This information will be very useful in breeding strategies aimed at improving the overall vigor of crop plants in different climatic regions. The main aim of this research to dissect the molecular mechanisms by which a novel plant gene naturally serves to protect plants from cellular death. We have cloned the gene named *lls1* (*lethal leafspot 1*) from maize and found that this gene encodes a novel but yet unknown function. In the absence of this single gene the plant will die before reaching maturity. We will now confirm that this novel gene function has a cell protective role in other plants in particular in another model plant species (*Arabidopsis*) where the function of this gene can be studied more thoroughly. We will also investigate how the protective role of this gene is turned on and off following wounding of the plant. At the same time we will determine the exact compartment within plant cells in which this gene provides its protective function. These experiments should reveal the molecular mechanism of action of this gene and thus aid in the strategic development of plants exhibiting increased stress resistance.

2000-01372 Gordon Conference on Mitochondria and Chloroplasts
Stern, D.
Gordon Research Conferences; West Kingston, RI 02892-0984
Grant 00-35301-9139; $5000; 1 Year

The Gordon Conference accept relatively few participants and feature open discussions and a convivial atmosphere, where internationally-known scientists mix freely with young investigators, students and postdoctoral fellows. The conference funded by this proposal covers mitochondria and chloroplasts, two essential energy-producing compartments in eukaryotic cells. When mutations occur that affect the expression of mitochondrial or chloroplast genes, the result is often reproductive of fatal disorders, both in animals and plants. The conference will highlight areas where recent progress has been most impressive, and areas that were under-represented in the last conference in this series. The subjects include organelle signaling networks, mitochondrial mutations leading to disease phenotypes, basic gene expression mechanisms and recent advances in the genetic engineering of chloroplasts. The fundamental research will impact agriculture because it is becoming increasingly popular to localize new traits in plants in the chloroplast, and knowledge of gene expression mechanisms is essential to this process. The reason that chloroplasts are increasingly being used for genetic engineering is that chloroplasts genes are transmitted through the female parent in most species, rather than through the pollen,

and thus the risk of spreading transgenes to exotic species is reduced. USDA funds are being specifically used to fund the attendance of U.S. plant scientists at this meeting, supporting a mix of young scientists and more established ones. Some of these scientists, although carrying out state of the art research, would not be able to attend the meeting without these supplements.

2000-01379 mRNA Targeting to Subdomains of the Endoplasmic Reticulum: The Role of RNA Binding Proteins

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Grant 00-35301-9081; $210,000; 3 Years

The storage proteins dictate the protein quality of plant seeds and its value as a food source. Rice accumulates prolamines and glutelins, the two major storage protein classes. Although the prolamine and glutelin mRNAs are translated on the endoplasmic reticulum (ER), they are not distributed stochastically on this membrane complex. Instead, prolamine mRNAs are highly enriched on the ER membranes that delimit the prolamine protein bodies, whereas glutelin mRNAs predominant on the ER that are randomly distributed in the cytoplasm. Our results to date indicate that this spatial separation of the storage protein mRNAs on the distinct ER membranes is due in part to the fact that the prolamine RNAs are transported directly from the nucleus to the surface of the prolamine protein bodies. Several RNA binding proteins, which may participate in the transport and/or anchoring of prolamine RNAs to the protein bodies have been identified. These include a 120 kD RNA binding activity, which was isolated from an enriched cytoskeleton-protein body fraction, and EF1A and DnaJ homologues, which were identified to specifically interact with prolamine RNA sequences as assessed by yeast three hybrid system. In this grant application, we propose to determine the possible roles of these RNA binding proteins in prolamine RNA transport and/or anchoring using a variety of molecular, biochemical, genetic and cellular approaches. Information from these studies will be invaluable in efforts to increase
the quality and quantity of seed proteins as a food and in using plants as phytofactories for the manufacture of novel proteins.

2000-01504 Cross Incompatibility between Teosinte and Maize

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Grant 00-35301-9012; $271,000; 3 Years

Pollen of annual Mexican teosintes generally is able to fertilize maize successfully, and the F1 hybrids are fully fertile. Nevertheless, the occurrence of hybrid plants under field conditions is uncommon to rare. The maize growing at some sites is unable to fertilize ovules of its teosinte counterpart. We propose: (i) to analyze genetically the compatibility between 12 sympatric maize and teosinte populations, (ii) to characterize organization of genes within a region of chromosome 4 that confers incompatibility between silks of a particular teosinte collection and pollen of dent corn, and (iii) to determine whether this failure is due to a specific rejection (i.e., pistil-pollen incompatibility) or to an absence of components necessary for normal function (i.e., pistil-pollen incongruity). This teosinte barrier to crossing is readily transferable to corn. In principle, the barrier could be used to isolate one category of corn varieties or hybrids from contamination by others. For example, it could protect strains intended for human consumption from undesired strains such as those containing transgenes.

2000-01469 Genetic and Molecular Characterization of Genes That Influence Abscission and Cell Separation

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Grant 00-35301-9085; $253,000; 3 Years

This research will focus on the characterization of mutants affecting cell separation in *Arabidopsis*. Processes associated with cell separation occur in a variety of developmental contexts including lateral and adventitious root development, seedling elongation, pollen dehiscence, and pod shatter. Plants also shed entire organ systems such as leaves, flowers, and fruits in a specialized cell separation process known as abscission. *Arabidopsis* is an excellent model plant for this study due to the variety of mutant collections, the sequencing of the genome, the numerous mapping markers and facile transformation. We have already identified twelve delayed floral organ abscission mutants and are currently cloning several of these genes. We are also determining the genetic interactions between these genes using breeding techniques. In addition, we are using an alternative PCR-based approach in which we are searching for mutants with disruptions in cell wall hydrolytic enzymes. We have identified seven "knockout" and are determining the role of these genes as well as the genetic interactions with our other identified mutants. This broad-based approach will allow us to develop a model for genes regulating abscission and other processes of cell separation. Early pod shatter and premature leaf, floral, or fruit drop can result in severe economic loss due to poor quality as well as reduced yields. We believe that this research can potentially lead to the development of new and improved methods of controlling fruit, floral, and leaf abscission in multiple crops.

2000-01375 Dissection of the Ubiquitin-Conjugation Cascade in *Arabidopsis*

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Grant 00-35301-9040; $170,000; 2 Years

Much of plant physiology, growth, and development is controlled by the selective degradation of intracellular proteins. To understand how plants degrade proteins, we are studying a major proteolytic pathway involving the highly conserved, 76-amino acid protein, ubiquitin. In this pathway, chains of ubiquitin are covalently attached to proteins targeted for breakdown. These chains then serve as recognition signals for selective degradation of the target protein by the 26S proteasome, a large proteolytic complex specific for ubiquitinated intermediates. Using *Arabidopsis thaliana* as a model, we will investigate the selectivity of the pathway through a molecular characterization of the important recognition steps that determine which proteins should be modified by ubiquitin. This conjugation involves various combinations of ubiquitin conjugating enzymes or E2s working in concert with ubiquitin protein ligases or E3. We and others have recently shown that numerous isoforms of E2s and E3s exist in plants. *A. thaliana* bearing mutations in individual E2 and E3 genes will be isolated and their phenotypes studied to identify times in *Arabidopsis* growth and development that require these factors. The targets of these enzymes will then be determined by both genetic and biochemical strategies. Collectively, the data will help reveal how plants selectively degrade proteins and provide strategies for controlling protein breakdown when it interferes with agricultural productivity. Ultimately, we may also be able to enhance our ability to ectopically express foreign proteins in plants by redesigning them to avoid ubiquitin-mediated turnover.

2000-01475 Chloroplast mRNA Stability and Translation

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Photosynthesis, the process that converts light energy into chemical energy, occurs within intracellular compartments of plant cells called chloroplasts and is essential for both plant and animal nutrition. Chloroplast contain their own set of genes and gene expression apparatus, yet these organelles are dependent upon nuclear genes for their function. Some nuclear genes control photosynthesis by regulating the expression of chloroplast genes. In the green alga *Chlamydomonas reinhardtii*, a model photosynthetic organism, genetic studies have identified nucleus-encoded regulatory proteins that are required for chloroplast mRNA stability and translation. These regulatory proteins function by interacting with the 5' leader of a single species of chloroplast mRNA. We are using *petD* (photosynthetic electron transport) as a model chloroplast gene, which encodes an essential photosynthetic protein, to investigate mechanisms that control gene expression. The *petD* mRNA 5' leader contains three essential regulatory elements that promote mRNA stability and translation. The aim of this project is to understand the critical sequences and structures of these RNA elements and how they interact with regulatory proteins to promote gene expression. Results are expected to provide insights into nuclear-chloroplast interactions which control photosynthesis. Beyond its contribution to basic knowledge, our research should suggest ways of optimizing expression and function of foreign genes in plant chloroplasts.